Supporting Information

Materials and Methods

Oligonucleotides Preparation

DNA/RNA oligonucleotides used in this study were designed and obtained commercially from Beijing Genomics Institute (BGI) or Integrated DNA Technologies (IDT). Sequences and abbreviations are listed in Table 1. They were dissolved (according to the supplier's instruction) with ultra – pure nuclease-free distilled (Invitrogen) water to a concentration of 100 μM, quantified by NanoDrop1000 spectrophotometer (Thermo-Scientific) and stored at -20 ^{o}C before experiment.

Circular Dichroism (CD) Spectroscopy

The CD spectroscopy was performed using Jasco J-1500 CD spectrophotometer and a 1-cm path length quartz cuvette (Hellma Analytics) was employed in a volume of 2 mL. Samples with 5 μM DNA/RNA (final concentration) were prepared in 10 mM LiCac (pH 7.0) and 150 mM KCl/LiCl. Each of the DNA/RNA samples were then thoroughly mixed and denatured by heating at 95 °C for 5-minutes and cooled to room temperature for 15-minutes for renaturation. The DNA/RNA samples were excited and scanned from 220 – 310 nm at 25 °C and spectra were acquired every 1 nm. All spectra reported were average of 2 scans with a response time of 2 s/nm[1, 2]. They were then normalized to molar residue ellipticity and smoothed over 5 nm[3]. All data was analyzed with Spectra Manager™ Suite (Jasco Software).

Thermal melting monitored by UV spectroscopy

Samples were prepared to a concentration of 10 mM LiCac buffer, 150 mM salt (KCl/LiCl) and 5 μM DNA/RNA, with a total volume of 2 mL. For the concentration dependent experiments, the samples were prepared with DNA/RNA concentration ranged from 1 – 50 μM. Each of the samples was mixed thoroughly and heated at 95 °C for 5 minutes so as to denature the DNA/RNA. It was then cooled for 15-minutes at room temperature for renaturation. All UV melting experiments were performed on an Agilent Cary 100 UV-Vis Spectrophotometer, using 1-cm path length quartz cuvette. Before the experiment started, the sample block was first flushed with dry N_2 gas and cooled down to 5 °C for 5-minutes. After the sample solutions were loaded to the cuvettes, they were sealed with 3 layers of Teflon® tape to prevent vaporization at high temperature. The samples were scanned from 5 to 95 °C with a temperature incremental rate of 0.5 ˚C/min. The temperature was hold at 95 °C for 5-minutes before a reversed scan was performed, scanning from 95 to 5 °C with a rate of 0.5 °C/min. The unfolding and folding transitions in both scans were monitored at 295 nm.

Raw data obtained were subtracted by the blank solutions, which contains the same concentrations of LiCac buffer (pH 7.0) and corresponding salt only. It was then smoothed over 11 nm and its first derivative was plotted in Microsoft Excel. The final melting temperature was obtained by averaging the melting temperatures in the forward and reversed scans.

Thermodynamics Calculation

The oligos' G4 folding enthalpy and entropy were calculated from the 5 mM KCl UV melting curves[4]. The raw UV-melting curves were first normalized into fraction folded (θ), and the enthalpy (ΔH) and entropy (ΔS) were calculated according to the following equations:

$$\text{Folding Constant } (K_a) = \frac{[\text{G-quadruplex folded}]}{[\text{single strand DNA or RNA}]} = \frac{\theta(T)}{1-\theta(T)}$$

$$\Delta G = -RT\ln(K_a) = \Delta H - T\Delta S$$

$$\ln(K_a) = \frac{-\Delta H}{RT} + \frac{\Delta S}{R}$$

, where T = temperature expressed as Kelvin, θ(T) = fraction folded at a particular temperature and R = gas constant = 8.3144598 J mol^{-1} K^{-1}. A linear correlation with slope = $\frac{-\Delta H}{R}$ and y-intercept = $\frac{\Delta S}{R}$ can be obtained at the melting curve region in the plot of $\ln(K_a)$ against 1/T. and therefore, the ΔH (kJ mol^{-1}) and ΔS (J $K^{-1}mol^{-1}$) can be determined.

Native polyacrylamide gel electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was conducted using 10% PAGE (19:1 acrylamide: bis-acrylamide) containing 50 mM KCl (final concentration) was used in the gel polymerization. Oligonucleotides were 5'- labelled with fluorescein (FAM) and 0.5 µM was mixed with 10 mM LiCac buffer (pH 7.0) and 50 mM KCl and annealed at 95^{o}C for 5 minutes and then cooled immediately at 4^{o}C for one hour. Then 40% sucrose solution (1:1 sample: sucrose) and 1 µL of 6X gel loading dye (New England BioLabs® inc., #B7022S) was added to aid visualization. 5 µL was loaded to the wells and run at 100V for 100 minutes at 4^{o}C. Gel was scanned using FUJIFilm FLA-9000 and processed by MultiGuage software.

Fluorescence Spectroscopy

Fluorescence spectroscopy was performed using HORIBA FluoroMax-4 and a 1-cm path length quartz cuvette (Hellma Analytics) was used with a volume of 2 mL. Samples with 5 µM DNA/RNA were prepared in 10 mM LiCac (pH 7.0) and 150 mM KCl/LiCl. The samples were then denatued at 95 °C for 5-minutes and cooled to room temperature for 15 minutes for renaturation. For the measurement of intrinsic fluorescence of G-quadruplexes, the samples are excited at 260 nm and the emission spectra were acquired from 300 – 500 nm, as previously described[2]. Spectra were acquired every 2 nm at 25 ˚C. The bandwidth of the entrance and exit slits was 5 nm. All data were smoothed over 5 nm. Results here are from three independent experiments and analyzed using Microsoft Excel.

Table S1. Thermodynamics data of dG_3T and 24 bulged oligos calculated from their UV melting curves in 5mM KCl.

Oligos	Sequence	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔG_{298K} (kJ mol^{-1})	ΔG_{310K} (kJ mol^{-1})
dG_3T	GGGTGGGTGGGTGGG	-398.9	-1104.9	-69.6	-56.4
dG_3T-AB-1	G**A**GGTGGGTGGGTGGG	-203.1	-617.5	-19.1	-11.7
dG_3T-AB-2	GG**A**GTGGGTGGGTGGG	-133.1	-407.5	-11.7	-6.8
dG_3T-AB-3	GGGTG**A**GGTGGGTGGG	-127.6	-391.4	-11.0	-6.3
dG_3T-AB-4	GGGTGG**A**GTGGGTGGG	-130.2	-398.7	-11.4	-6.6
dG_3T-AB-5	GGGTGGGTG**A**GGTGGG	-161	-493.8	-13.8	-7.9
dG_3T-AB-6	GGGTGGGTGG**A**GTGGG	-136.9	-420.3	-11.7	-6.6
dG_3T-AB-7	GGGTGGGTGGGTG**A**GG	-119.5	-366.1	-10.4	-6.0
dG_3T-AB-8	GGGTGGGTGGGTGG**A**G	-306.1	-922	-31.3	-20.3
dG_3T-CB-1	G**C**GGTGGGTGGGTGGG	-343	-1023.3	-38.1	-25.8
dG_3T-CB-2	GG**C**GTGGGTGGGTGGG	-254.4	-762.4	-27.2	-18.1
dG_3T-CB-3	GGGTG**C**GGTGGGTGGG	-354.6	-1061.1	-38.4	-25.7
dG_3T-CB-4	GGGTGG**C**GTGGGTGGG	-375.9	-1127.9	-39.8	-26.3
dG_3T-CB-5	GGGTGGGTG**C**GGTGGG	-361.1	-1080.4	-39.1	-26.2
dG_3T-CB-6	GGGTGGGTGG**C**GTGGG	-351.3	-1053.2	-37.4	-24.8
dG_3T-CB-7	GGGTGGGTGGGTG**C**GG	-243.9	-731.3	-26.0	-17.2
dG_3T-CB-8	GGGTGGGTGGGTGG**C**G	-244.8	-732.5	-26.5	-17.7
dG_3T-TB-1	G**T**GGTGGGTGGGTGGG	-324.6	-971.5	-35.1	-23.4
dG_3T-TB-2	GG**T**GTGGGTGGGTGGG	-251	-754.8	-26.1	-17.0
dG_3T-TB-3	GGGTG**T**GGTGGGTGGG	-192.1	-580.3	-19.2	-12.2
dG_3T-TB-4	GGGTGG**T**GTGGGTGGG	-138.8	-424.7	-12.2	-7.1
dG_3T-TB-5	GGGTGGGTG**T**GGTGGG	-248.5	-750.2	-24.9	-15.9
dG_3T-TB-6	GGGTGGGTGG**T**GTGGG	-194.9	-593	-18.2	-11.1
dG_3T-TB-7	GGGTGGGTGGGTG**T**GG	-119	-360.2	-11.7	-7.3
dG_3T-TB-8	GGGTGGGTGGGTGG**T**G	-274.3	-823	-29.0	-19.2

All oligos showed negative enthalpy and entropy, which indicates the folding of dG4s is exothermic and leads to a decrease in randomness. All ΔG in both 25 and 37 ˚C showed negative values, suggesting dG4 formations.

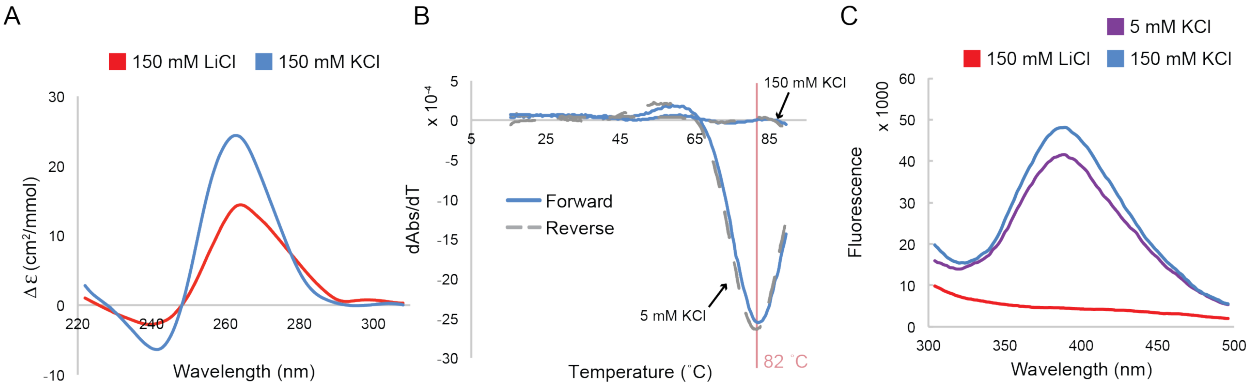

Figure S1. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262nm and ~240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be greater than 95 ˚C under 150 mM KCl condition. The melting temperature of the dG4 under 5 mM KCl was determined to be 82 ˚C. The dotted grey lines indicate the reverse scans. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH 7.0) and 5 mM KCl (Purple), 150 mM KCl (Blue) and LiCl (Red) at 25 ˚C. From this data, the dG4 forms and the λ_{em} were determined to be 386 nm under KCl condition.

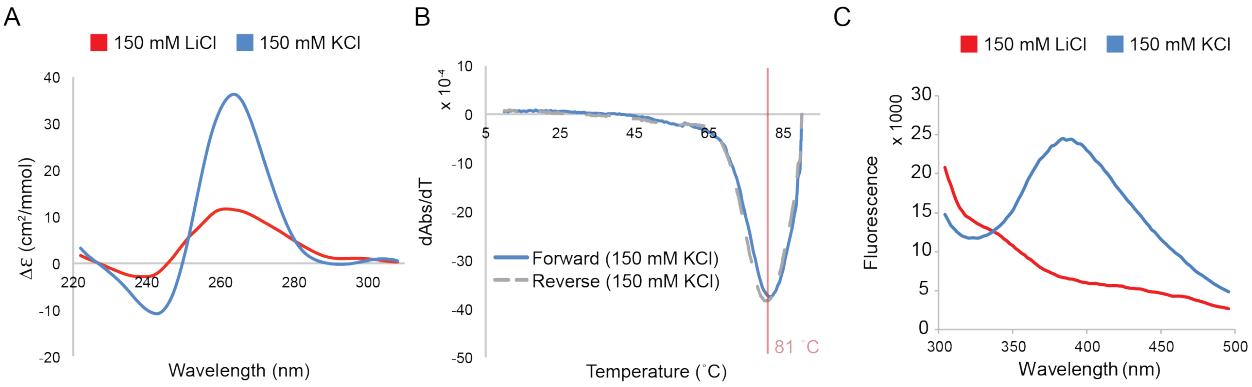

Figure S2. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-1.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-1 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) conditions. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-1 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the G-quadruplex was determined to be 81 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 386 nm under KCl condition.

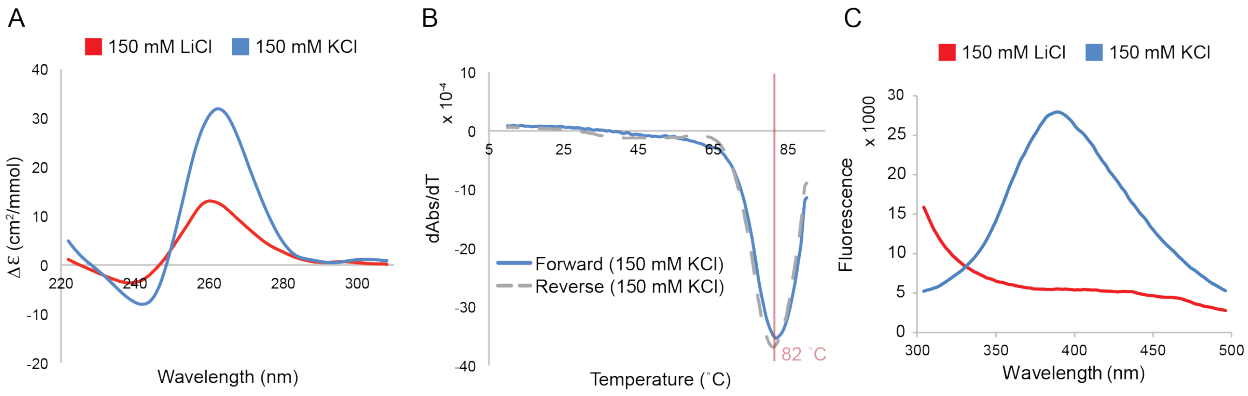

Figure S3. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-2.

From left to right: **A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-2 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) conditions. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-2 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 82 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

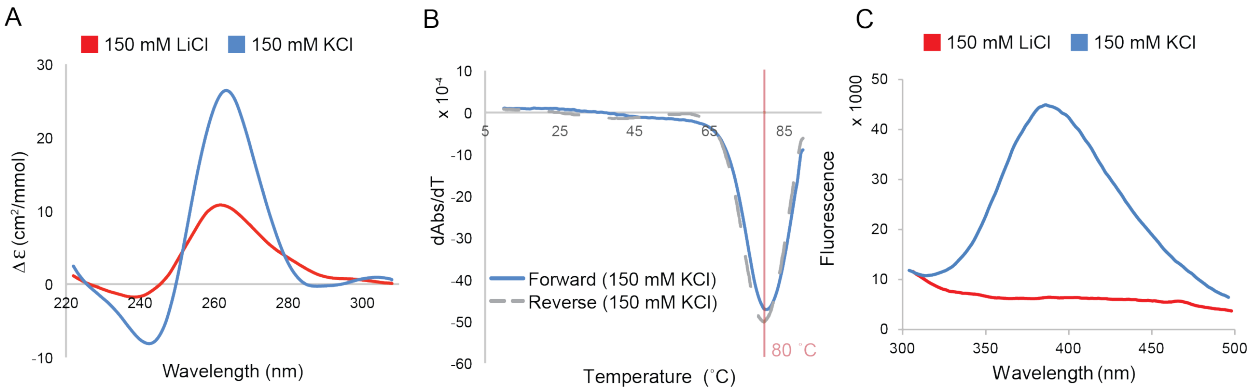

Figure S4. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-3 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-3 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 80 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 386 nm under KCl condition.

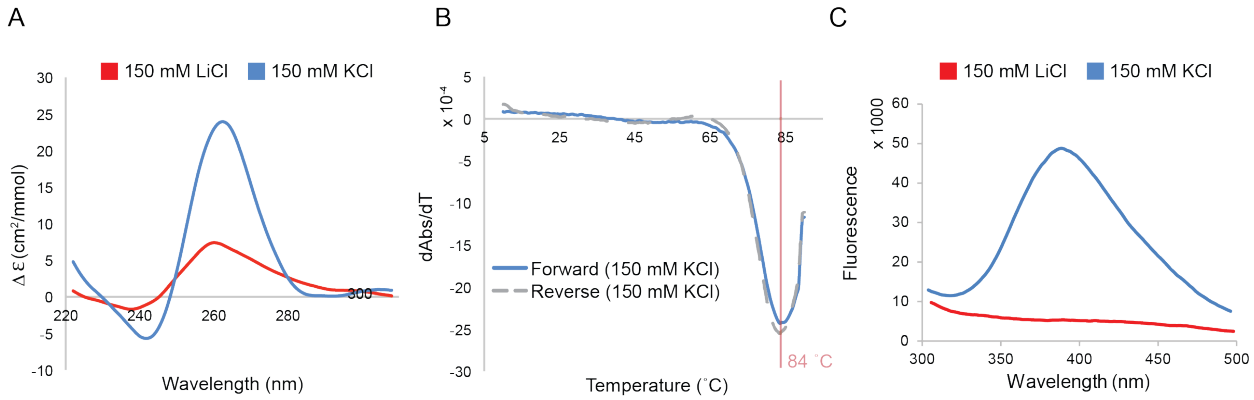

Figure S5. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-4.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-4 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-4 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

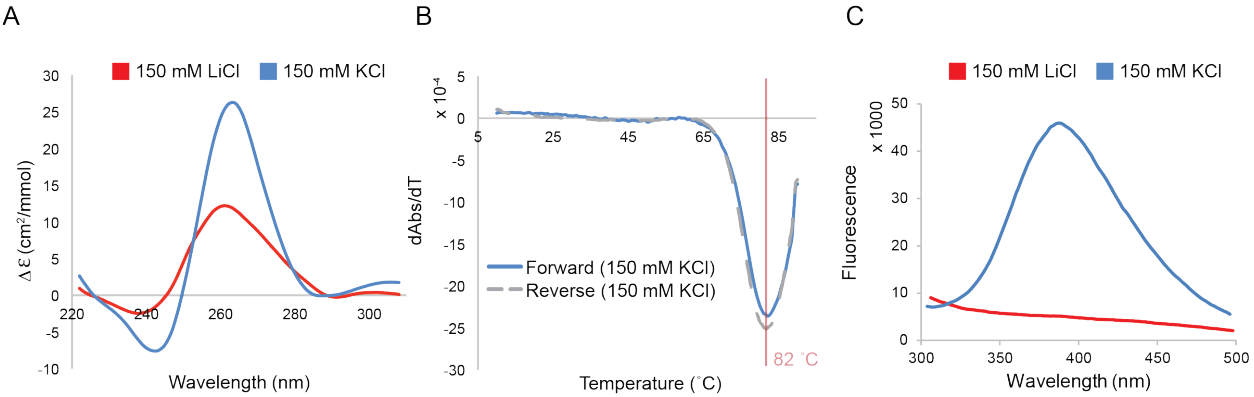

Figure S6. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-5.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-5 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red). The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-5 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 82 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{o}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

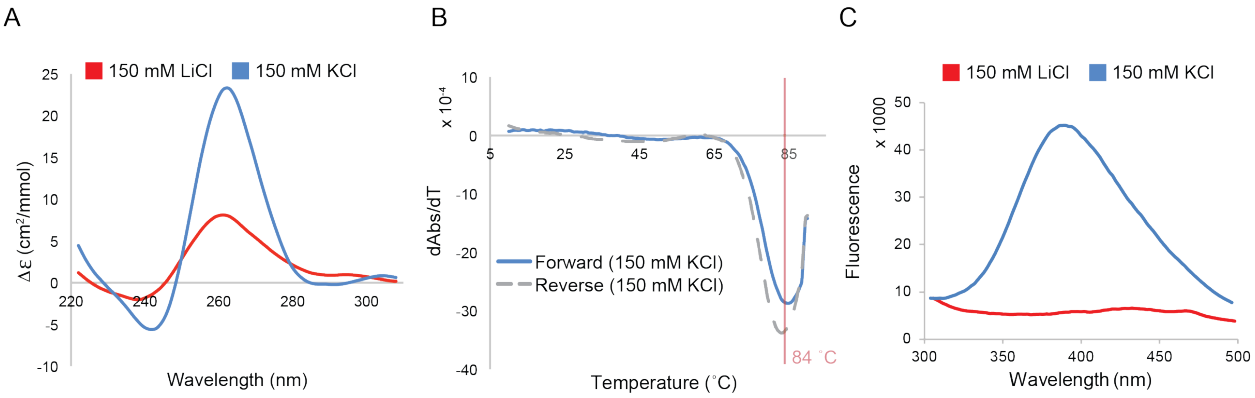

Figure S7. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-6.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-6 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-6 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 388 nm under KCl condition.

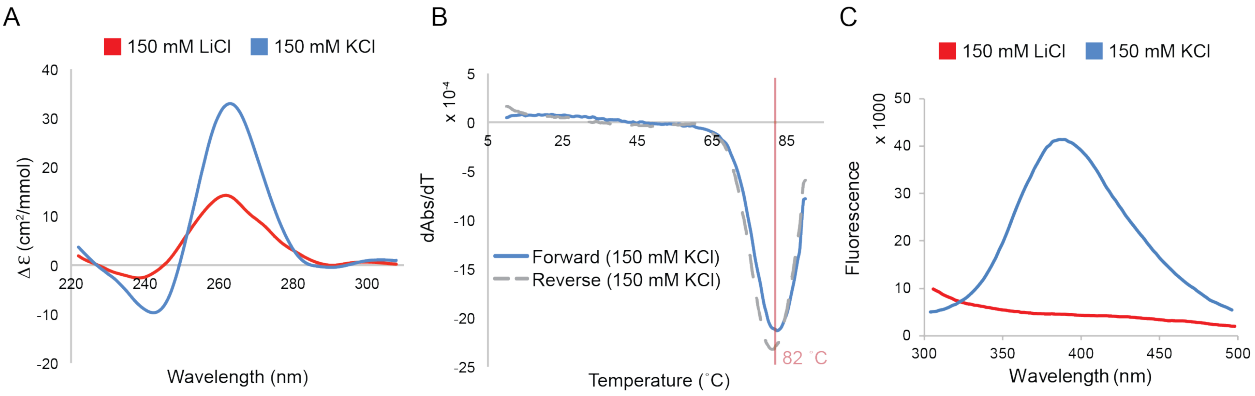

Figure S8. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-7.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-7 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-7 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 82 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 386 nm under KCl condition.

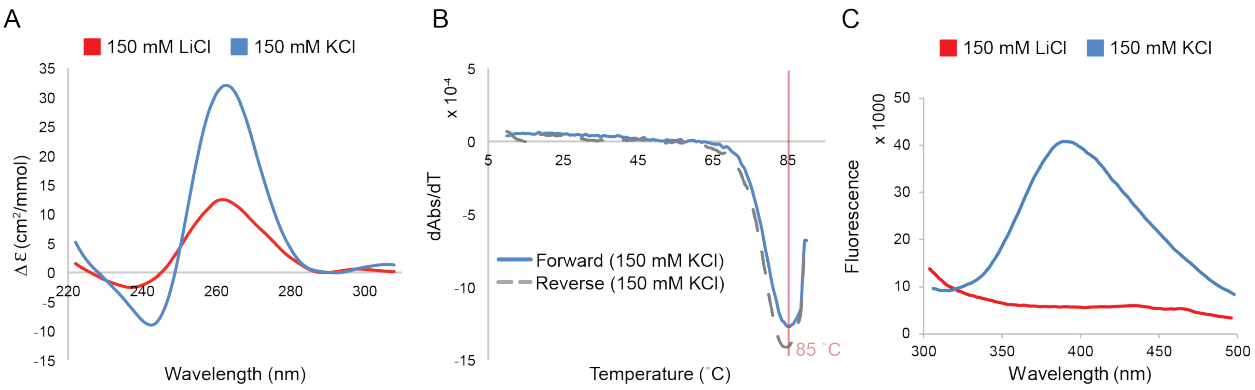

Figure S9. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-AB-8.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-AB-8 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-AB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 85 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

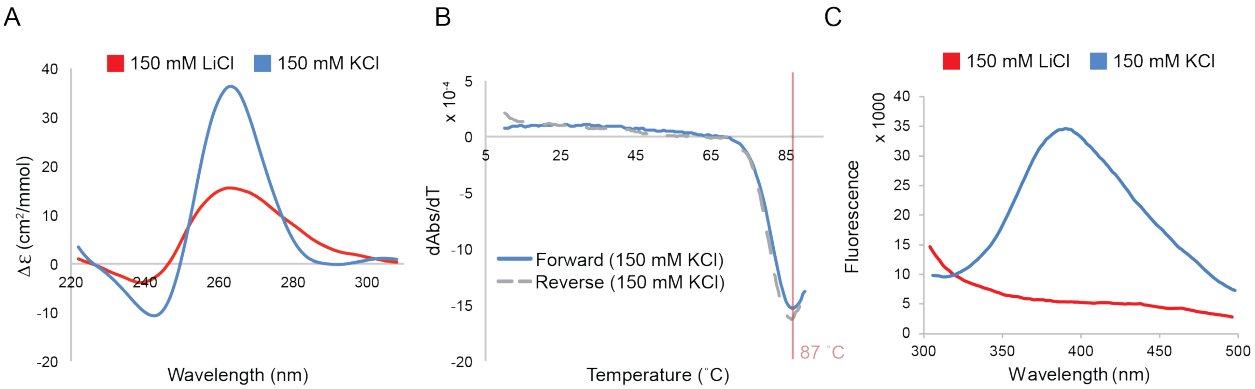

Figure S10. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-1.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-1 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-1 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 87 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 390 nm under KCl condition.

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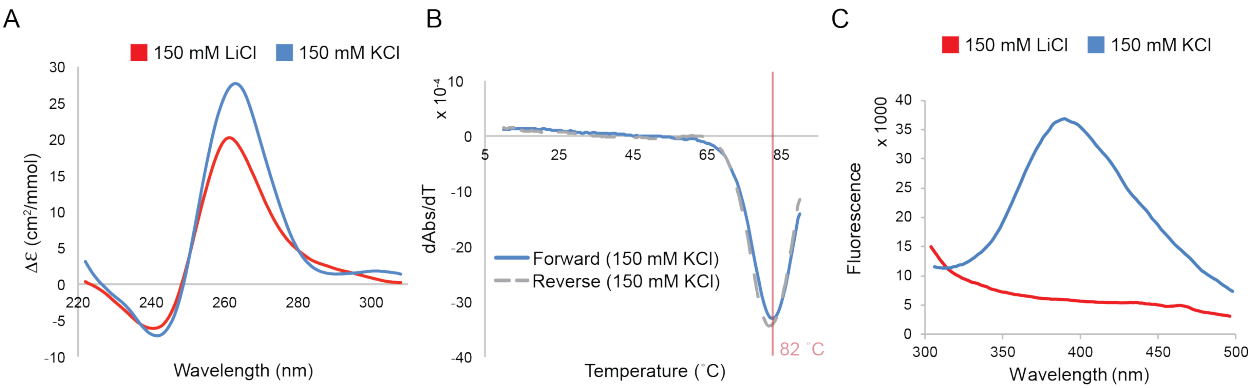

Figure S11. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-2.

From left to right: **(A)** CD detected titration, **(B)** UV detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-2 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-2 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 82 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

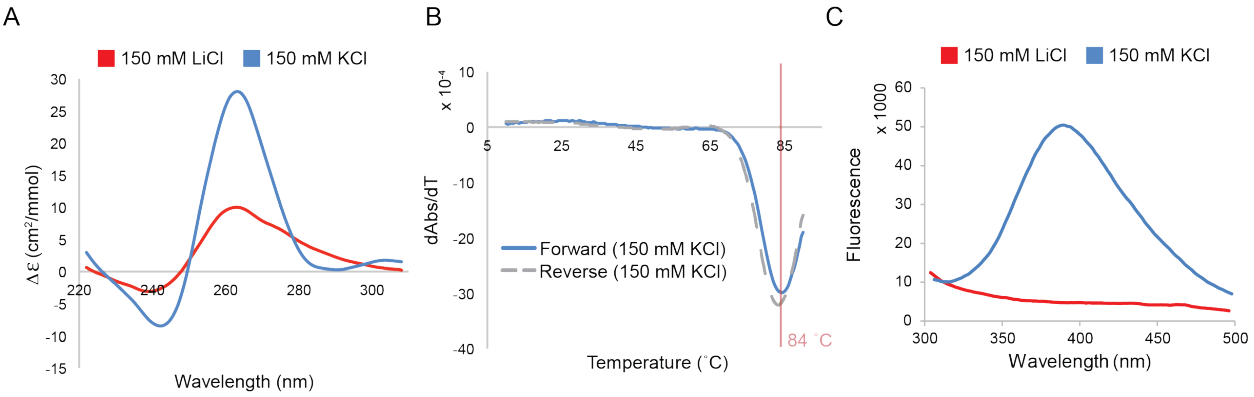

Figure S12. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-3 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-3 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

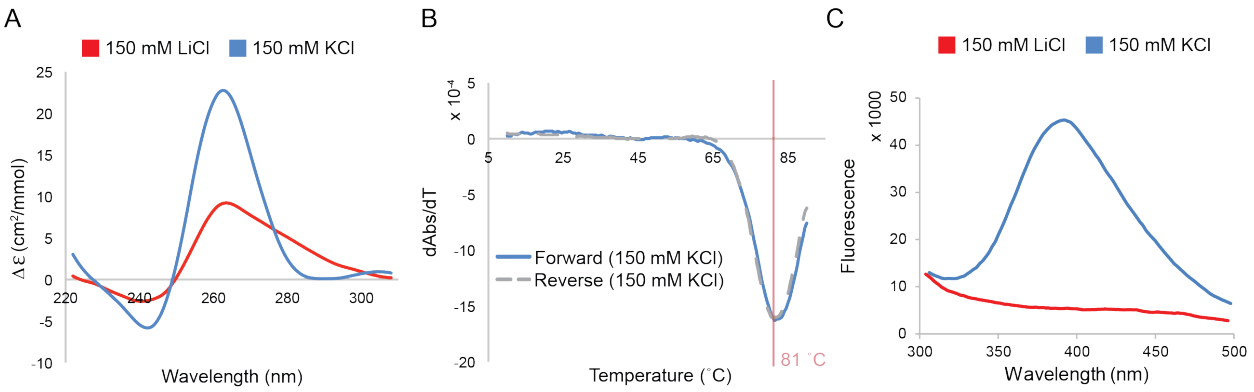

Figure S13. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-4.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-4 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-4 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 81 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 391 nm under KCl condition.

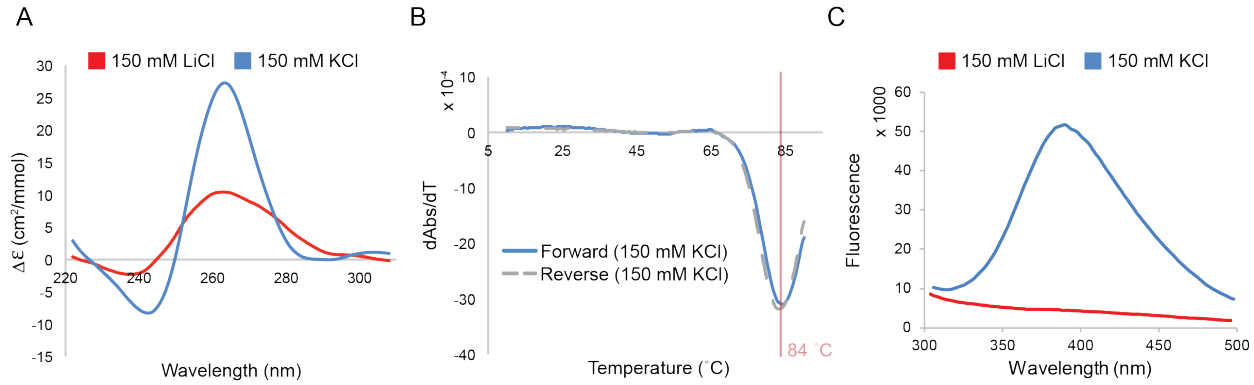

Figure S14. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-5.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-5 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-5 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

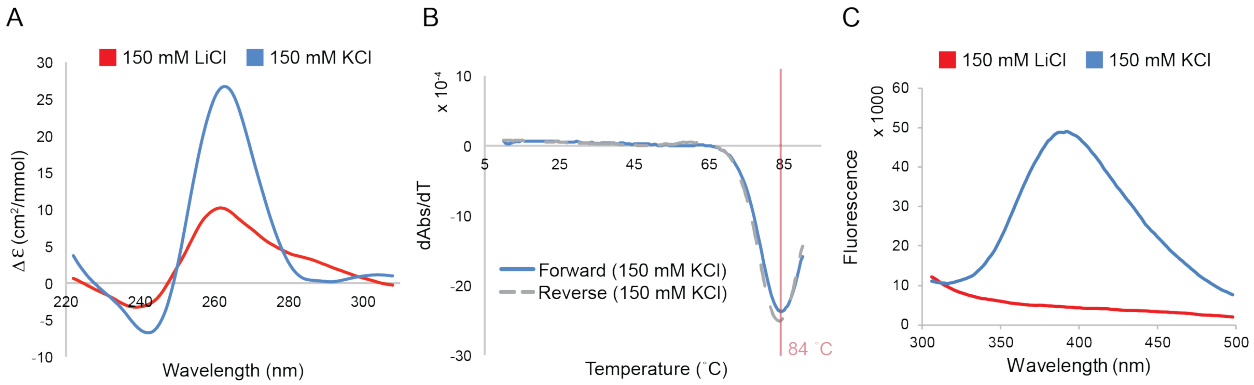

Figure S15. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-6.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-6 sequence at a DNA concentration of 5 μM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-6 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 390 nm under KCl condition.

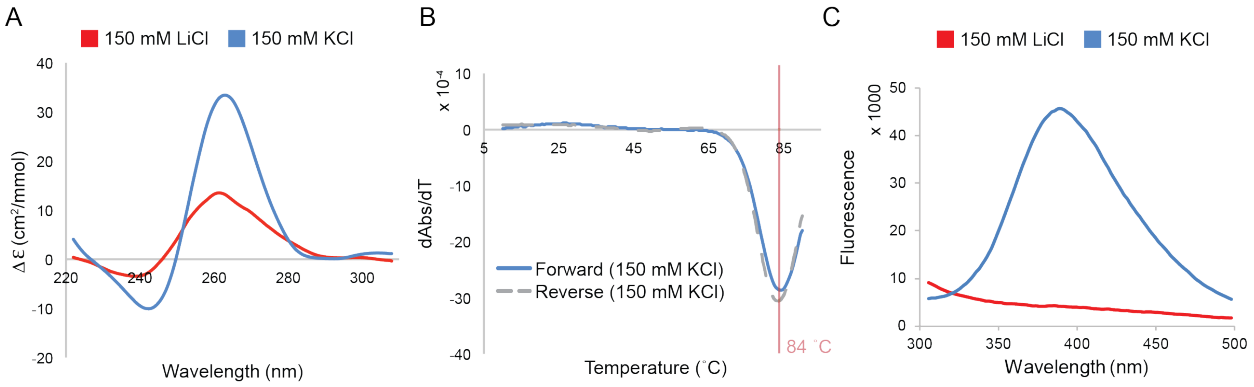

Figure S16. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-7.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-7 sequence at a DNA concentration of 5 μM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-7 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

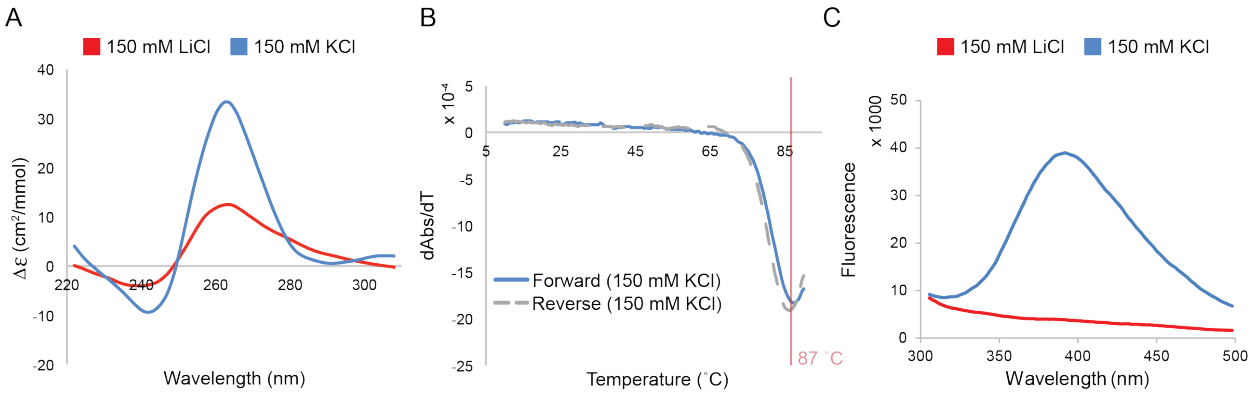

Figure S17. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-CB-8.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-CB-8 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-CB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 87 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 390 nm under KCl condition.

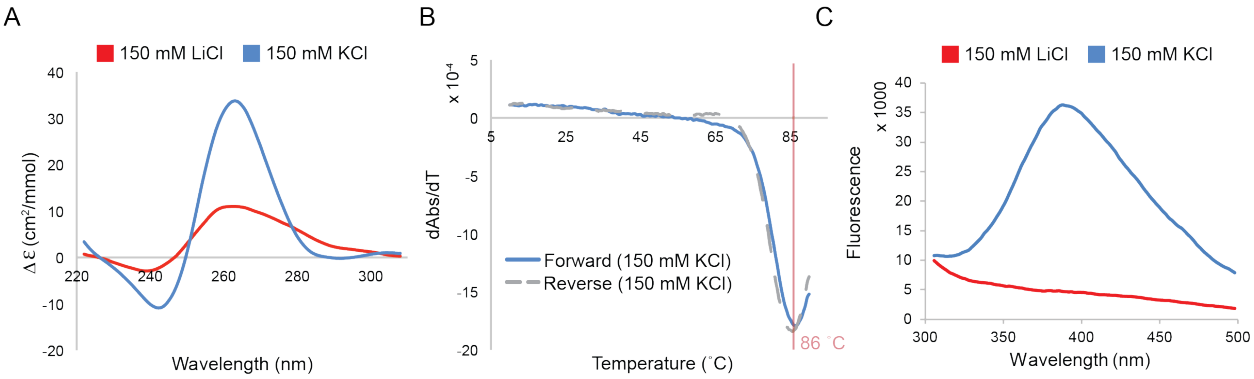

Figure S18. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-1.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-1 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-1 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 86 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

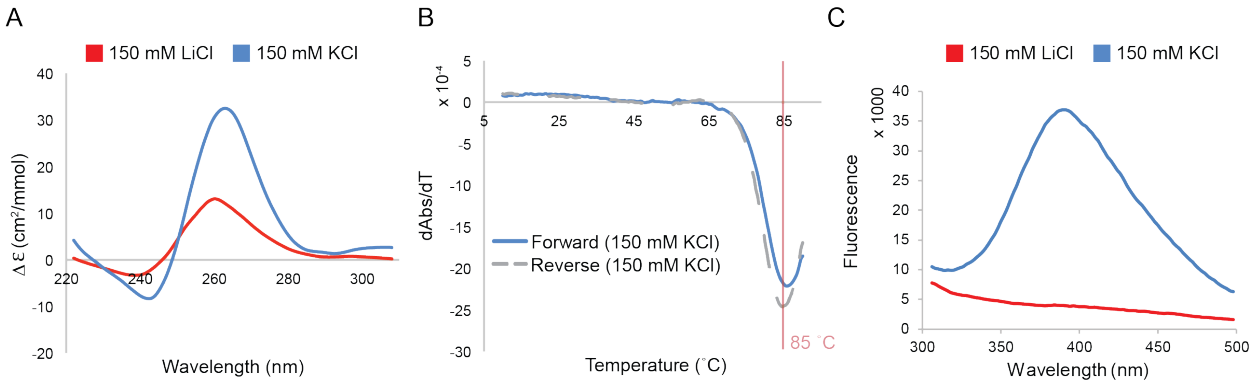

Figure S19. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-2.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-2 sequence at a DNA concentration of 5 µM. **(A)** CD – detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-2 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 85 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

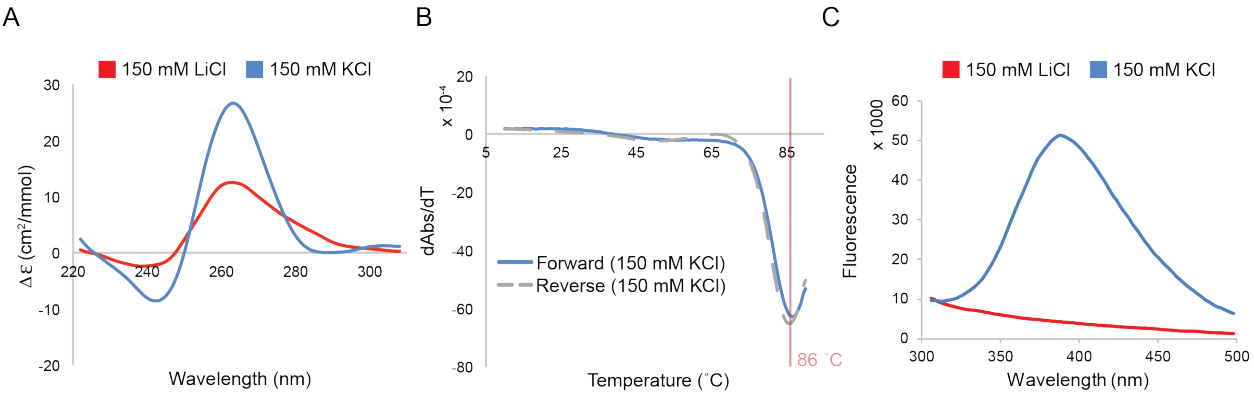

Figure S20. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-3 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-3 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 86 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 386 nm under KCl condition.

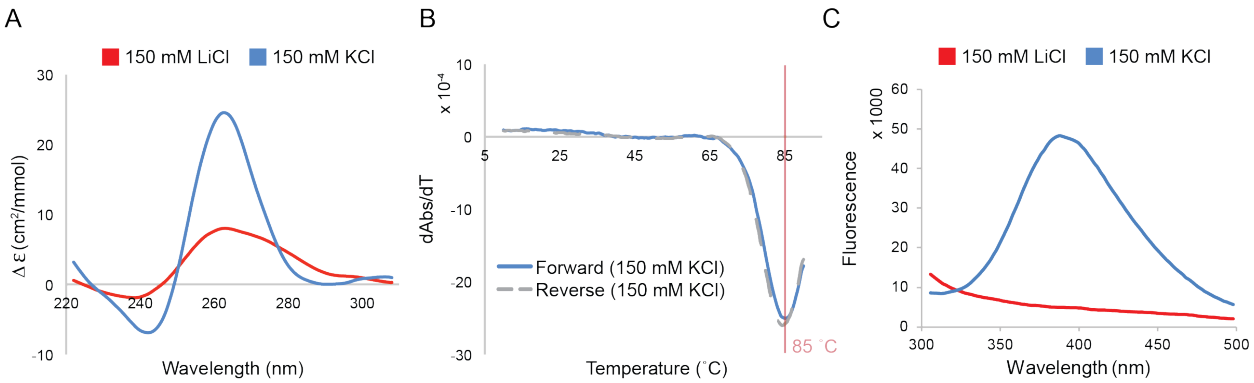

Figure S21. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-4.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-4 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-4 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 85 ˚C under 150mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 388 nm under KCl condition.

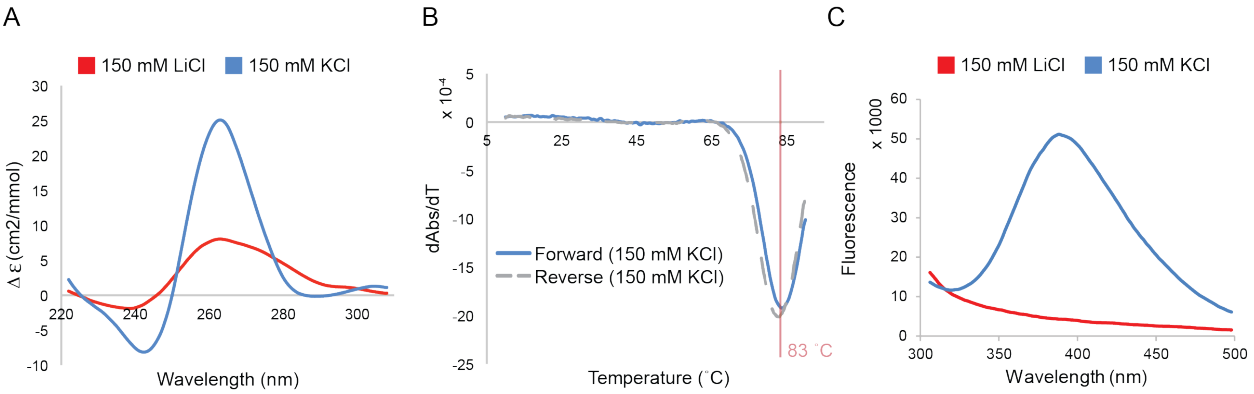

Figure S22. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-5.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-5 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-5 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 83 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 386 nm under KCl condition.

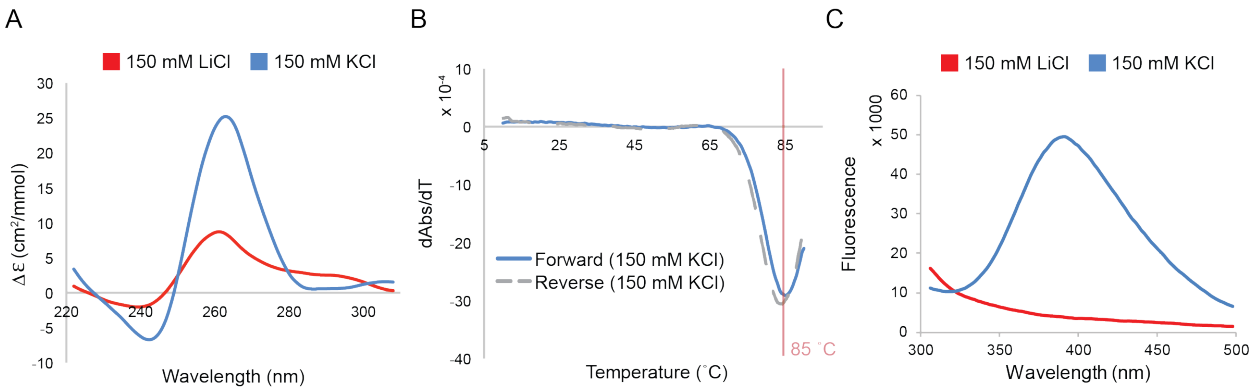

Figure S23. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-6.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-6 sequence at a DNA concentration of 5 μM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-6 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 85 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

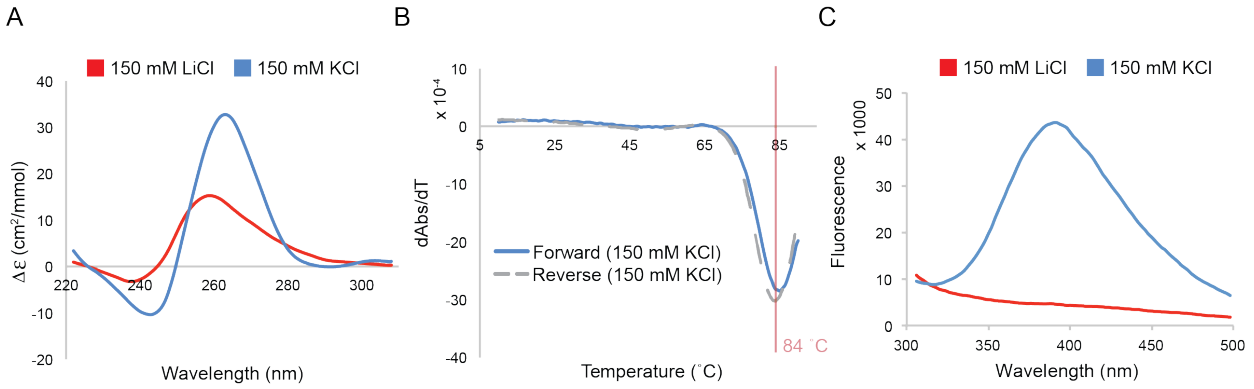

Figure S24. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-7.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-7 sequence at a DNA concentration of 5 μM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-7 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 84 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 389 nm under KCl condition.

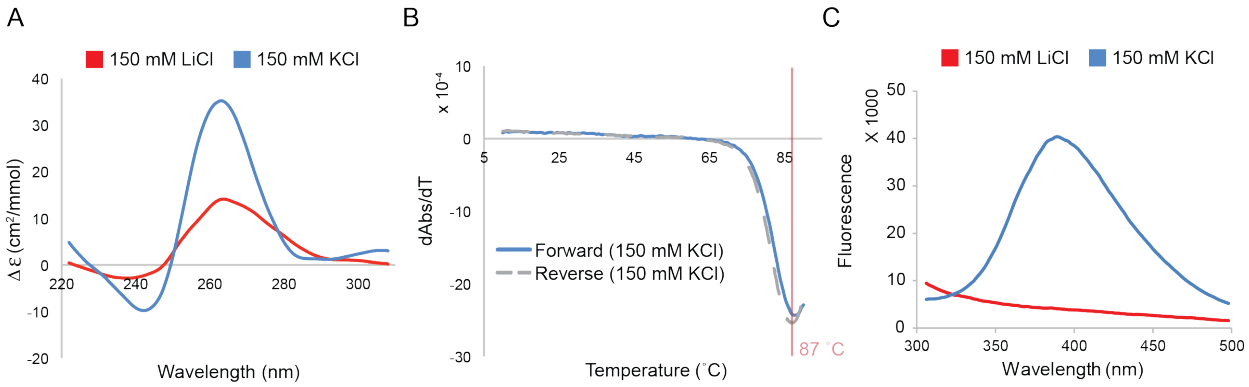

Figure S25. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of dG_3T-TB-8.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of dG_3T-TB-8 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 87 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 387 nm under KCl condition.

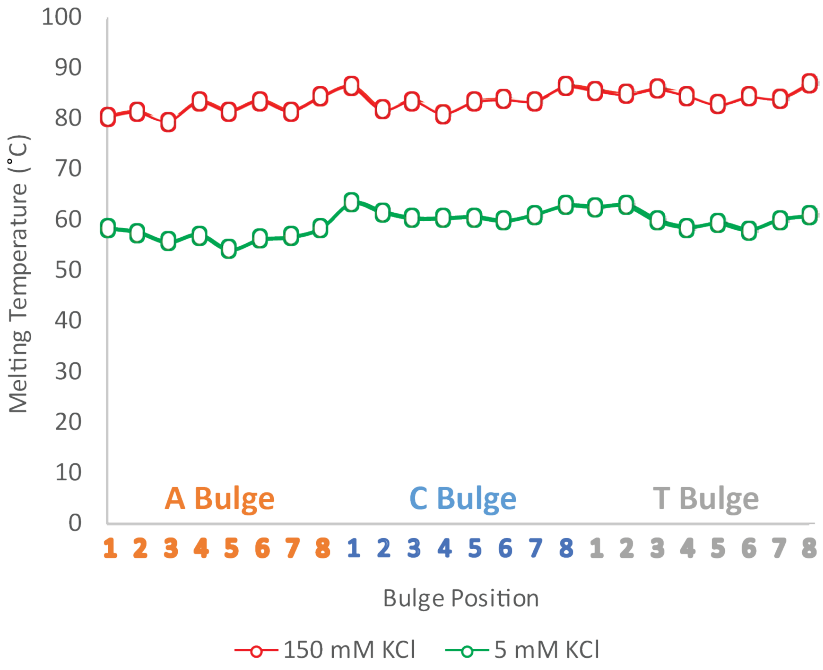

Figure S26. UV melting of 24 bulged oligos under 150 mM and 5 mM KCl.

The melting temperatures obtained from the UV-melting profiles of 24 bulged oligos under 150 mM (red) and 5 mM (green) KCl. In 150 mM KCl, the oligos with highest T_m was dG_3T-TB-8 (87 ˚C) and the lowest was dG_3T-AB-3 (80 ˚C). In 5 mM KCl, the oligos with highest T_m was dG_3T-CB-1 (64 ˚C) and the lowest was dG_3T-AB-5 (54 ˚C)

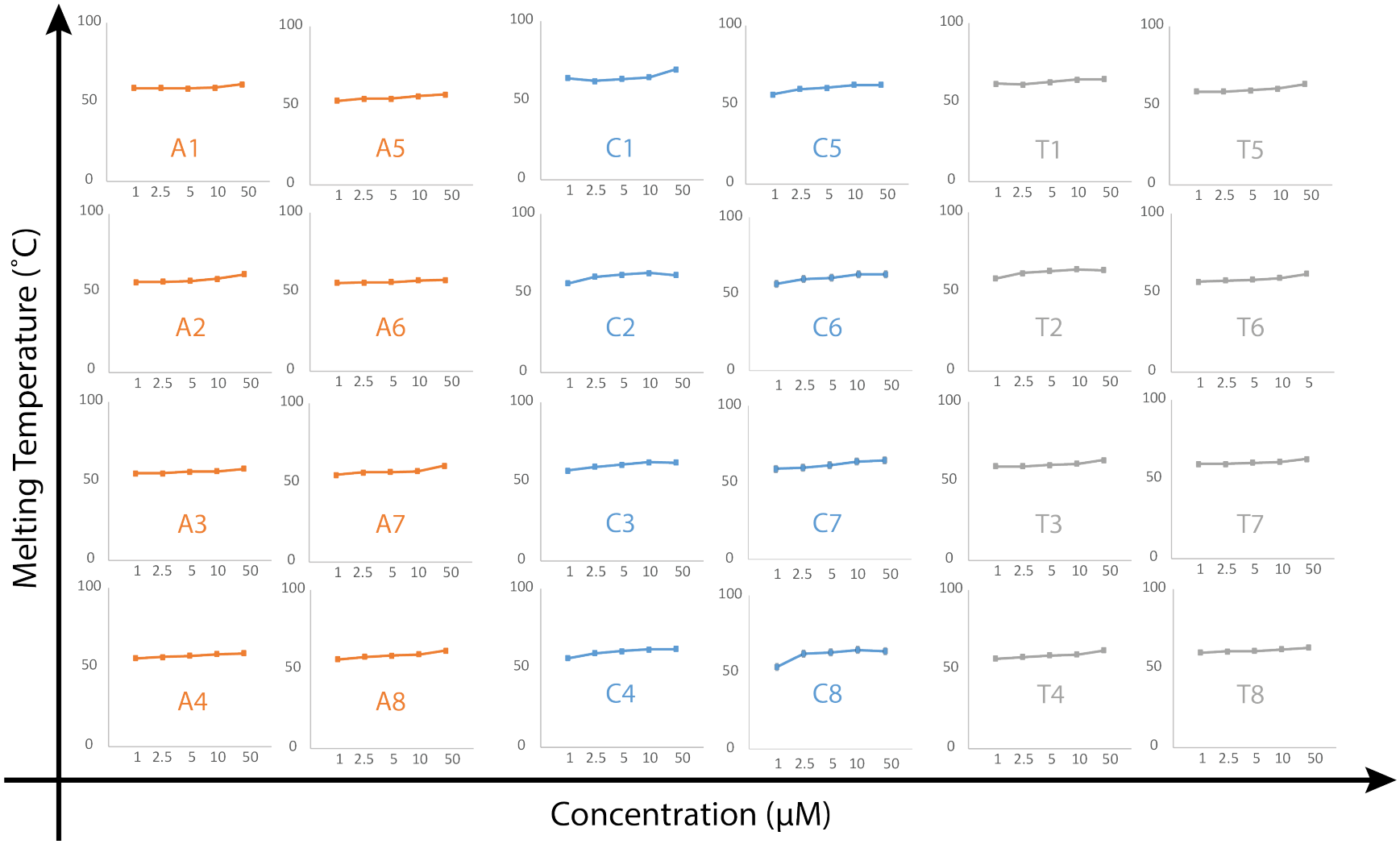

Figure S27. Concentration-dependent UV melting of 24 bulged oligos.
The melting temperatures obtained from the 5 mM KCl UV-melting profiles of 24 bulged oligos in this study in 1, 2.5, 5.0, 10.0 and 50.0 μM. It was observed that the melting temperature was independent of the oligonucleotide concentration, suggesting the G-quadruplex of the oligos are intramolecularly folded under our testing condition in the fluorescence assay at 5 μM.

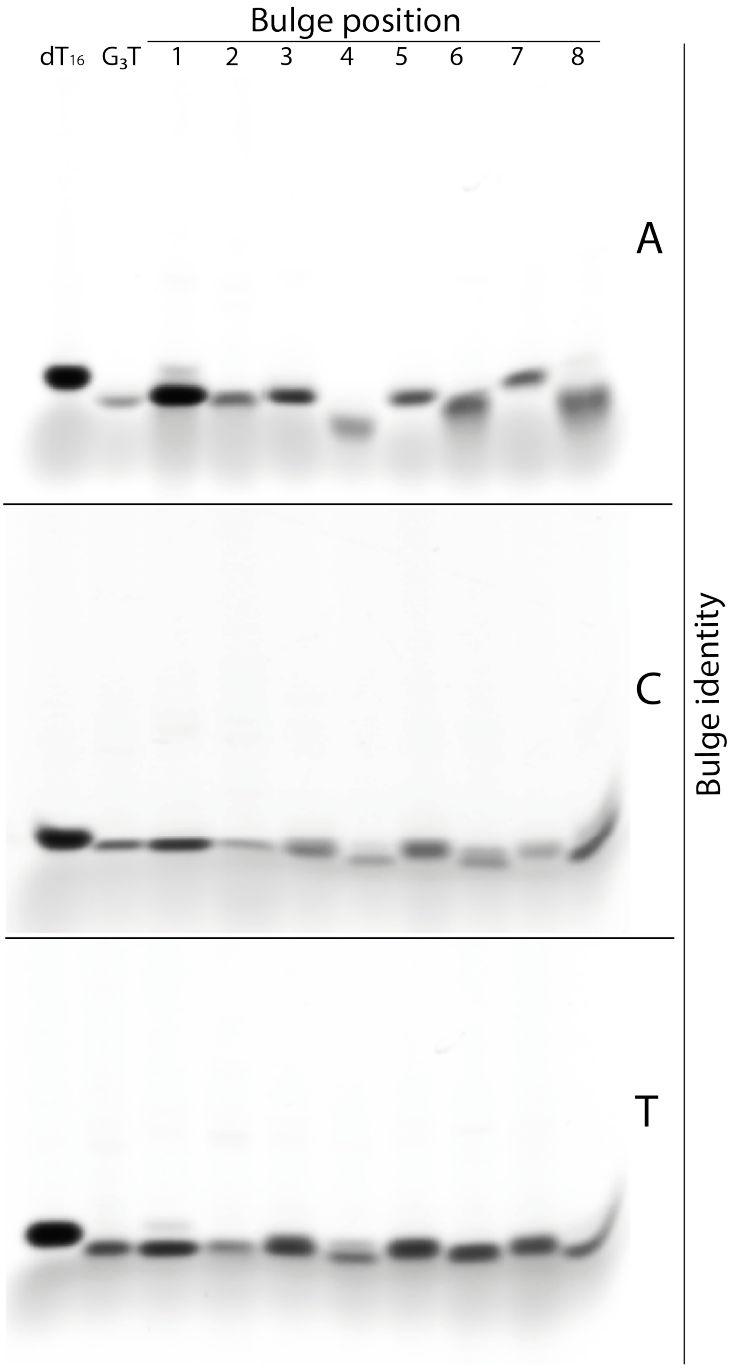

Figure S28. Native gel assay of dG_3T and 24 bulged dG4 oligonucleotides.
5'-FAM labeled samples (0.5 μM final concentration) were prepared in 50 mM KCl and 10 mM LiCac buffer (pH 7.0), loaded to a native 10% polyacrylamide gel containing 50 mM KCl and ran at 4 ^{o}C. A single stranded marker dT_{16} was used (first lane; from left to right); second lane was dG_3T; numbers 1 – 8 refers to the bulge positions of the bulged dG4s, while the letters A, C and T refers to the bulge identity of the bulged dG4s. As seen from the gel, a single band was detected for all oligos, and ran faster than the single stranded dT_{16} marker, which suggest the formation of intramolecular dG4s.

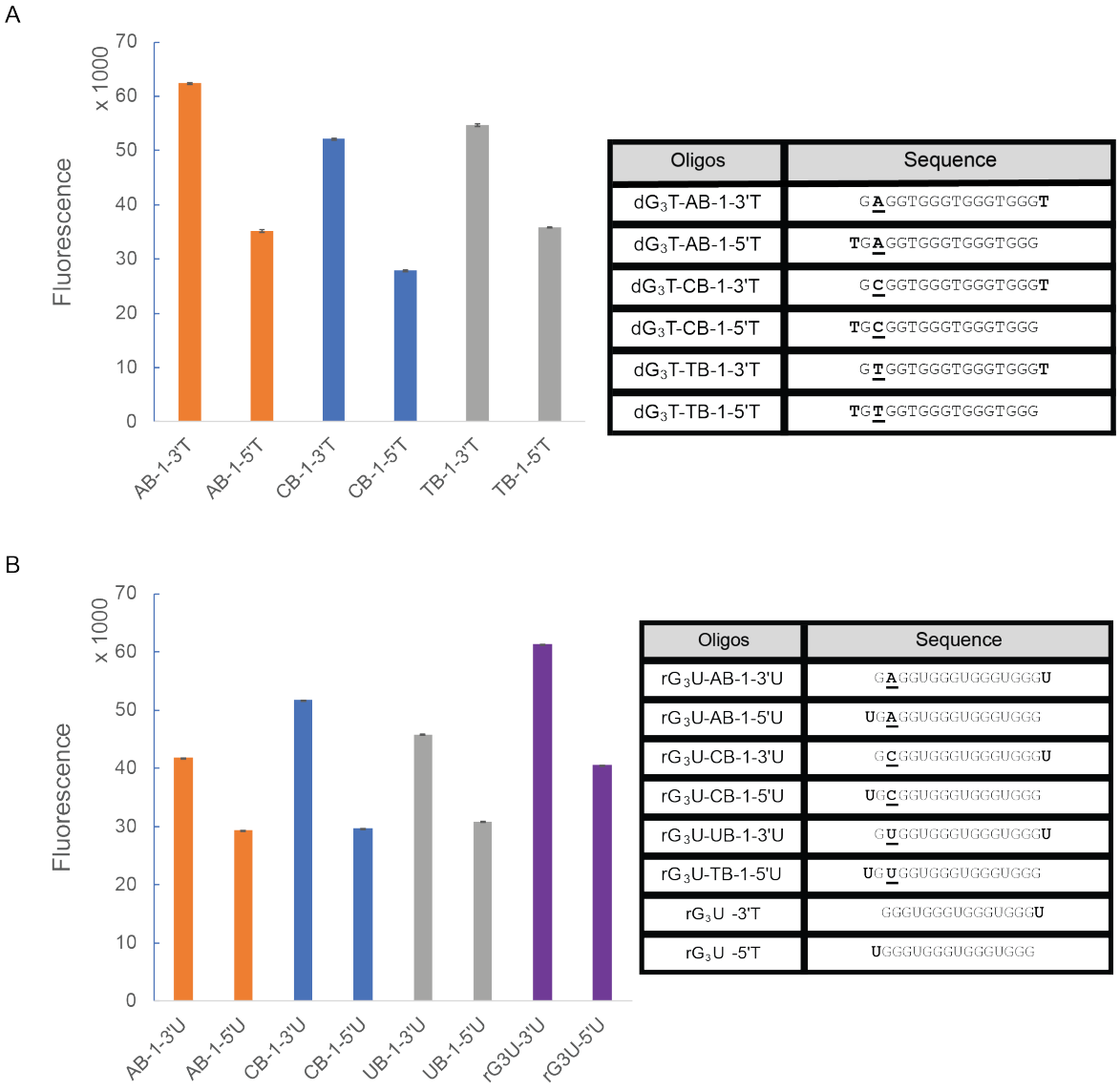

Figure S29. Intrinsic fluorescence intensity of T-dangling oligos with bulges at position N1.
From top to bottom: Intrinsic fluorescence intensity (in thousands) of T-dangling **(A)** DNA and **(B)** RNA oligos with bulge at position N1. All 3'T-dangling oligos, regardless of DNA or RNA, showed a stronger fluorescence than their 5'T-dangling counterparts, which is consistent with a previous study that investigated the dangling effect on dG_3T[5]. In our study, all 5'T-dangling oligos only showed a 50-70% of emission intensity of their 3'T-dangling counterparts.

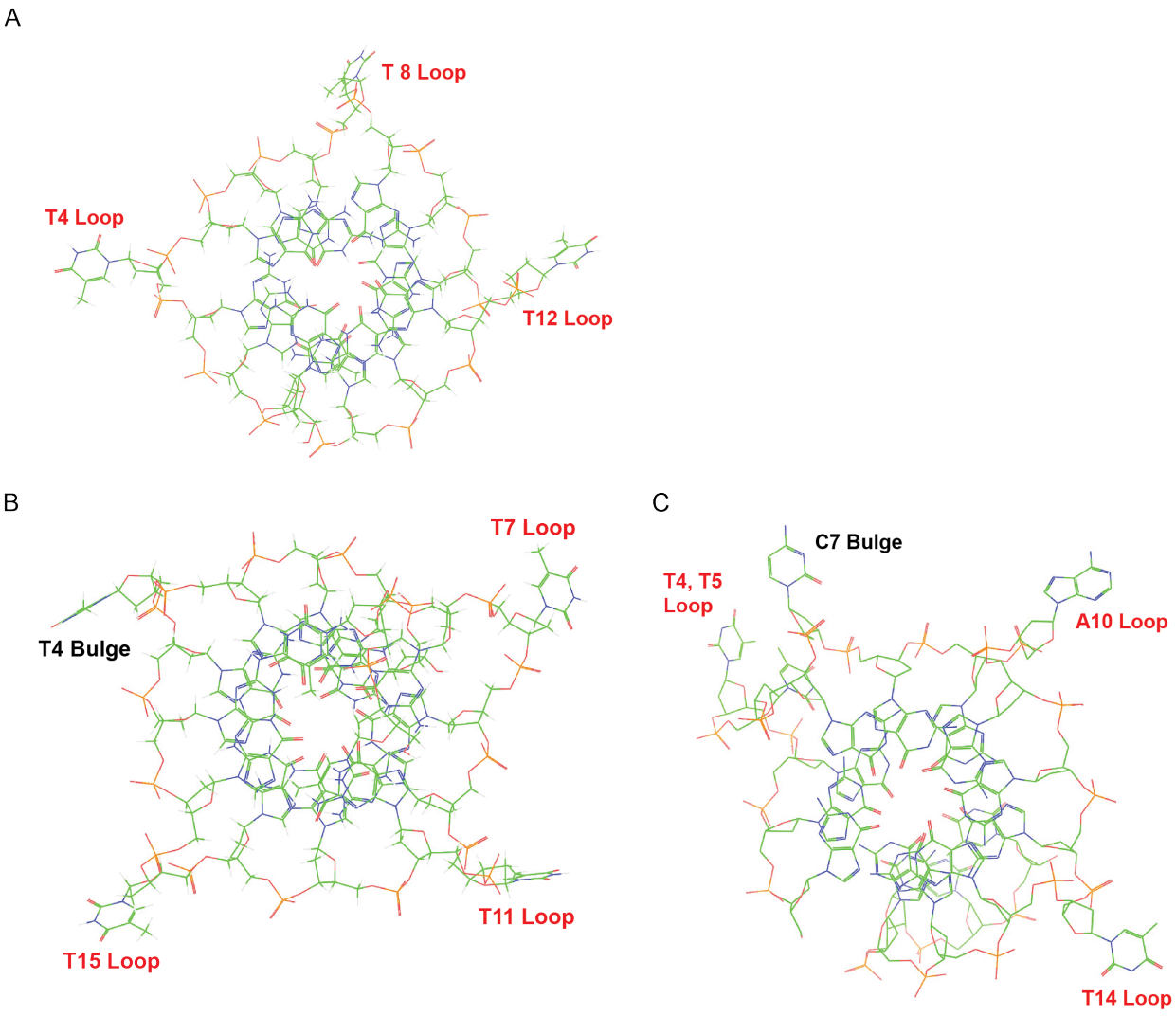

Figure S30. High-resolution structures of three G-quadruplexes that are related to this study. X-ray crystallography of dG4 oligos with sequences **(A)** GIGTGGGTGGGTGGGT (PDB ID= 2le6)[6], **(B)** TTGTGGTGGGTGGGTGGGT (PDB ID=2m4p)[7] and **(C)** GGGTTGCGGAGGGTGGGCCT (PDB ID= 5ua3)[8]. Only a monomer is shown for the structures in **(A)** and **(C)**. The 3 structures clearly showed that the nucleotides of both loop (Red font) and bulge (black font) were flipped out and did not interact with the remaining scaffold of the G-quadruplexes.

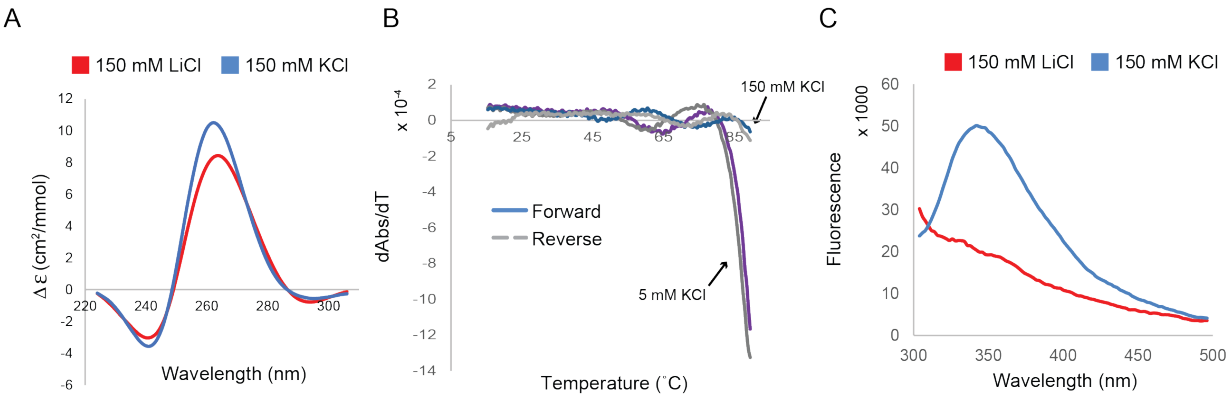

Figure S31. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of rG_3U. From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of rG_3U sequence at an RNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology rG4, suggesting that rG_3U forms a parallel topology rG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the rG4 was determined to be greater than 95 ˚C under 150 mM and 5 mM KCl conditions. Solid blue and purple lines indicate the forward scans respectively, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH 7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 342 nm under KCl condition.

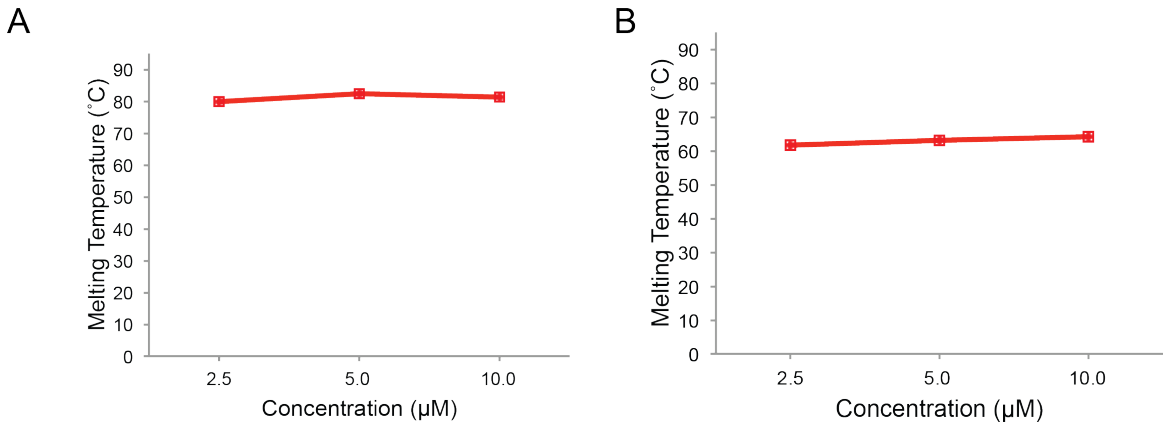

Figure S32. Concentration-dependent UV melting of hTR 1-20 dG4-CB-3 and hTR 1-20 rG4-CB-3. The melting temperatures obtained from the UV-melting profiles of **(A)** hTR 1-20 dG4-CB-3 and **(B)** hTR 1-20 rG4-CB-3 in 2.5, 5.0 and 10.0 µM respectively. It was observed that the melting temperatures were independent of the oligonucleotide concentration, suggesting that the G-quadruplexes of these 2 oligos are intramolecularly folded.

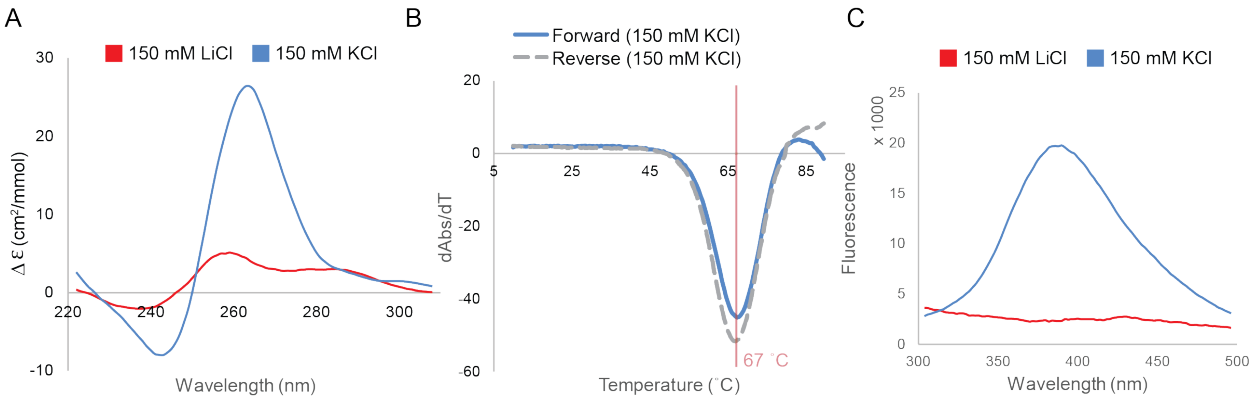

Figure S33. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of hTR 1-20 dG4-TB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of hTR 1-20 dG4-TB-3 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 67 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 390 nm under KCl condition.

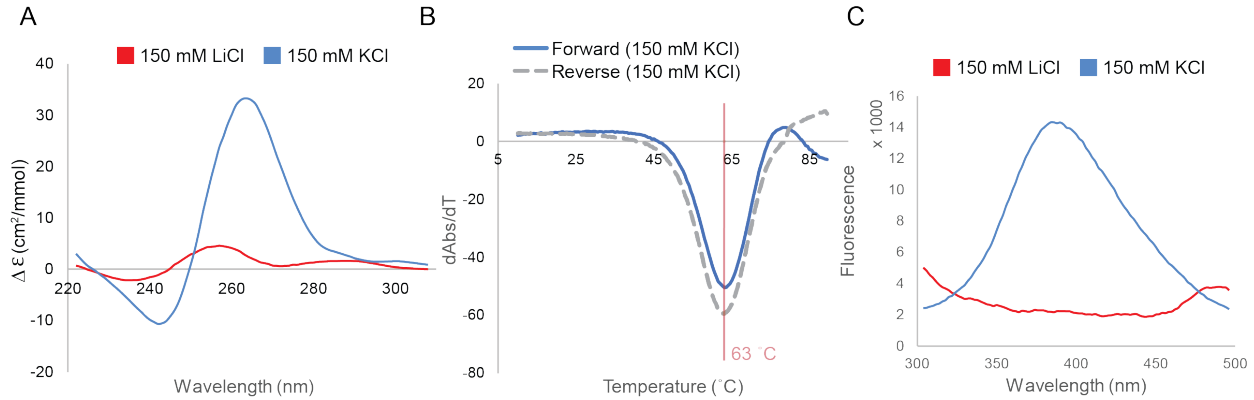

Figure S34. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of hTR 1-20 dG4-AB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of hTR 1-20 dG4-AB-3 sequence at a DNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 63 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 384 nm under KCl condition.

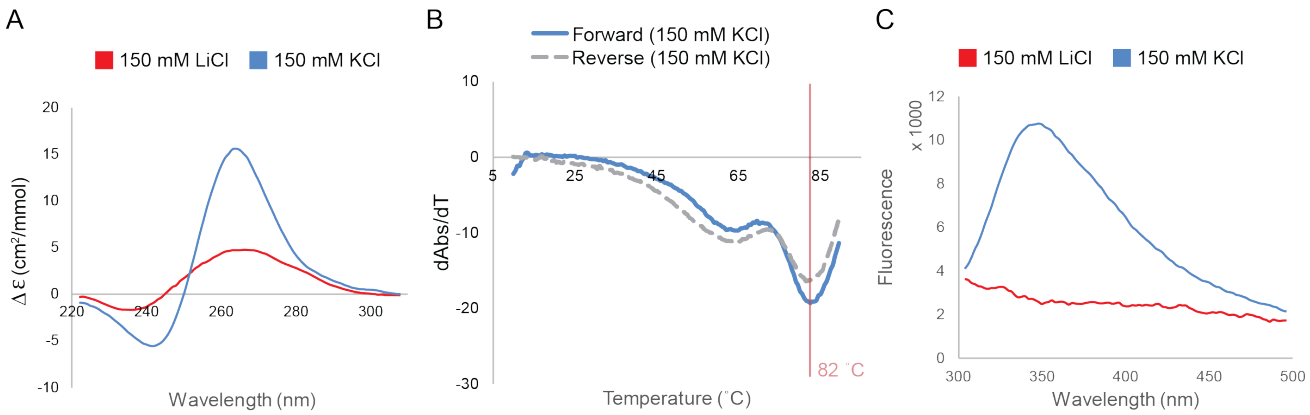

Figure S35. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of hTR 1-20 rG4-UB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of hTR 1-20 rG4-UB-3 sequence at an RNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 82 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 348 nm under KCl condition.

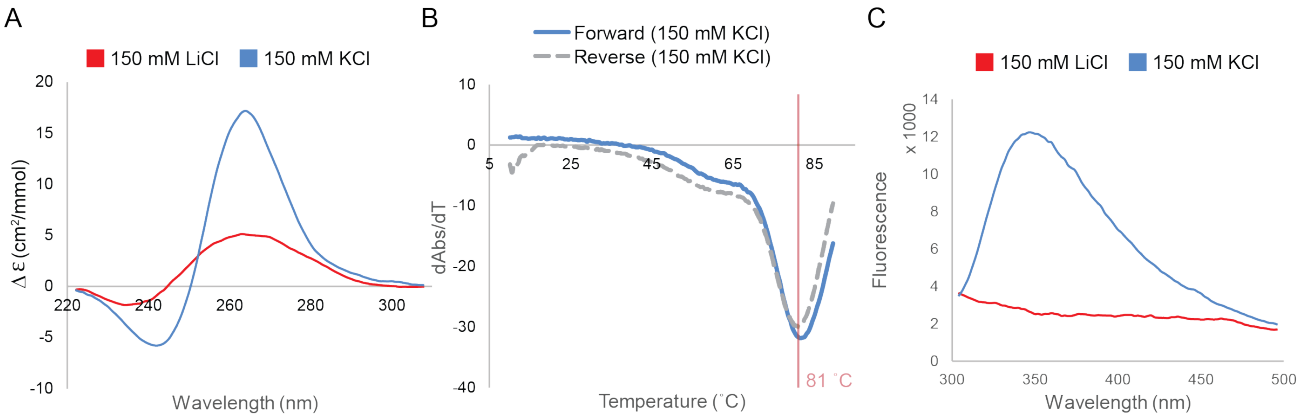

Figure S36. CD-detected titration, UV-detected melting and intrinsic fluorescence titration of hTR 1-20 rG4-AB-3.

From left to right: **(A)** CD-detected titration, **(B)** UV-detected melting and **(C)** intrinsic fluorescence titration of hTR 1-20 rG4-AB-3 sequence at an RNA concentration of 5 µM. **(A)** CD-detected titration scanned from 220 – 310 nm at 25 ˚C under KCl (Blue) and LiCl (Red) condition. The positive and negative peak at ~262 nm and 240 nm, as well as the K^+-dependent CD signal are signature of parallel topology dG4, suggesting that dG_3T-TB-8 forms a parallel topology dG4. **(B)** UV-detected melting monitored at 295 nm and the melting temperature of the dG4 was determined to be 81 ˚C under 150 mM KCl condition. Solid blue line indicates forward scan, and dotted grey line indicates the reversed scan. **(C)** Intrinsic fluorescence titration in 10 mM LiCac (pH7.0) and 150 mM KCl (Blue) and LiCl (Red) at 25 ^{0}C. From this data, the dG4 forms and the λ_{em} was determined to be 346 nm under KCl condition.

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